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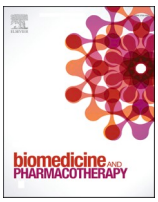

Review

# The role of hepatocyte-derived extracellular vesicles in liver and extrahepatic diseases

Shihui Li [a,b], Fang Cheng [a,b], Zhuan Zhang [a,b], Ruizi Xu [a,b], Honglei Shi [c,d,e,*], Yongmin Yan [a,d,e,**]

[a] Department of Laboratory Medicine, Wujin Hospital Affiliated with Jiangsu University, Changzhou 213017, China
[b] Department of Laboratory Medicine, School of Medicine, Jiangsu University, Zhenjiang 212013, China
[c] Wujin Hospital Affiliated With Jiangsu University, Changzhou Wujin People's Hospital, Changzhou Medical Center, Nanjing Medical University, Changzhou 213004, China
[d] Changzhou Key Laboratory of Molecular Diagnostics and Precision Cancer Medicine, Wujin Hospital Affiliated with Jiangsu University (Wujin Clinical College of Xuzhou Medical University), Changzhou 213017, China
[e] Wujin Institute of Molecular Diagnostics and Precision Cancer Medicine of Jiangsu University, Changzhou 213017, China

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ABSTRACT

Extracellular vesicles (EVs) are vesicle-like bodies with a double membrane structure that are released from the cell membrane or secreted by cells into the extracellular environment. These include exosomes, microvesicles, and apoptotic bodies. There is growing evidence indicating that the composition of liver cell contents changes following injury. The quantity of EVs and the biologically active substances they carry vary depending on the condition of the liver cells. Hepatocytes utilize EVs to modulate the functions of different liver cells and transfer them to distant organs via the systemic circulation, thereby playing a crucial role in intercellular communication. This review provides a concise overview of the research on the effects and potential mechanisms of hepatocyte-derived EVs (Hep-EVs) on liver diseases and extrahepatic diseases under different physiological and pathological conditions. Common liver diseases discussed include non-alcoholic fatty liver disease (NAFLD), viral hepatitis, alcoholic liver disease, drug-induced liver damage, and liver cancer. Given that NAFLD is the most prevalent chronic liver disease globally, this review particularly highlights the use of hepatocyte-derived EVs in NAFLD for disease progression, diagnosis, and treatment.

## 1. Introduction

Liver disease has emerged as a significant global public health concern, with the potential to progress to liver failure and mortality. Common liver ailments include fatty liver, viral hepatitis, and hepatocellular carcinoma, all of which stem from abnormalities in hepatocytes and other liver nonparenchymal cells. Interactions among hepatocytes, macrophages, and hepatic stellate cells (HSCs) are present in various liver diseases, yet the precise mechanism of these interactions remains unclear. Hepatocytes, the primary liver cells responsible for substance synthesis and drug/toxin metabolism, release mediators like cytokines, chemokines, and hormones during injury [1–3]. These mediators may act in a paracrine manner on adjacent cells, thereby influencing disease progression [4]. Notably, EVs have recently garnered attention for their potential role in regulating cell-to-cell communication and their association with numerous liver diseases.

EVs are membrane-bound vesicles released by cells, containing a diverse array of bioactive molecules including proteins, DNA, lipids, and metabolites [5,6]. EVs can be found in various biological fluids such as blood, saliva, urine, and amniotic fluid [7]. Importantly, the composition of EVs contents can be altered in response to pathological or stressful conditions [8]. Hepatocytes secrete EVs with varying components and functions depending on the physiological or pathological environment [9].

EVs has yielded significant discoveries and holds promise for advancing pathological diagnostics and treatments. While liver biopsy

* Corresponding author at: Wujin Hospital Affiliated With Jiangsu University, Changzhou Wujin People's Hospital, Changzhou Medical Center, Nanjing Medical University, Changzhou 213004, China.
** Corresponding author at: Department of Laboratory Medicine, Wujin Hospital Affiliated with Jiangsu University, Changzhou 213017, China.
*E-mail addresses:* dashi7810@163.com (H. Shi), yym@wjrmyy.cn (Y. Yan).

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remains the current gold standard for diagnosing and staging liver diseases, it is associated with risks of bleeding and sampling errors. Although imaging methods are commonly utilized, they may not accurately detect early signs of liver injury. EVs have emerged as potential biomarkers for diagnosing liver diseases due to alterations in their composition throughout disease progression. Previous studies have demonstrated that Hep-EVs have the ability to stimulate liver regeneration, suggesting their potential for therapeutic interventions [10]. Additionally, the unique biocompatibility and protective characteristics of EVs make them effective carriers for targeted treatments of liver diseases.

This article aims to explore the role of EVs released by hepatocytes in intercellular communication within different disease environments. It will also discuss the diagnostic and therapeutic implications of EVs, with a specific focus on the role of hepatocyte EVs in the context of NAFLD.

## 2. Overview of extracellular vesicles

### 2.1. Classification of extracellular vesicles

EVs are vesicle-like structures with a double-layer membrane that are either shed from the cell membrane or secreted by cells. They are classified into three types based on their production process, release pathway, size, content, and function [11]. The first type, exosomes (30–150 nm), are produced through the cell's endogenous pathway and transport various biological molecules such as proteins [12], lipids, and nucleic acids for intercellular communication and material delivery [13,14]. The second type, microvesicles (MV), are larger in size (100–1000 nm) and are formed by budding outward from the cell membrane, containing bioactive molecules like growth factors, cytokines, and signaling lipids. Microvesicles play roles in intercellular communication, immune regulation, and coagulation processes [15]. The third type, apoptotic bodies, are larger in size (1–5 μm) and are released by cells undergoing programmed cell death [16], carrying biological macromolecules such as cellular chromatin, enzymes, and organelles [17,18]. Additionally, some laboratories have also identified additional EVs subpopulations like migrasome and oncosome. [19,20].

Although exosomes, microvesicles, and apoptotic bodies are distinct in theory, current technology is unable to differentiate between them based on structure or function. Therefore, the International Society for Extracellular Vesicles recommends categorizing vesicles smaller than 200 nm as small extracellular vesicles and those larger than 200 nm as large extracellular vesicles [21]. Large extracellular vesicles are enriched in proteins associated with ribosome and RNA biogenesis, processing, and metabolism [22], while small extracellular vesicles are enriched in proteins related to cell-cell junctions, cell-matrix adhesion, exosome biogenesis mechanisms, and various signaling pathways [23]. Exosomes, being the smallest in size, most uniform, and with a complex composition, are considered to have the highest theoretical and application value among the three types of extracellular vesicles. Hence, exosomes have undergone extensive study and broad application. In academic literature and daily discourse, the terms exosomes and EVs are often used interchangeably. Consequently, this review will refer to all types of extracellular vesicles as EVs.

### 2.2. Mechanism of formation and composition of exosomes

The cytoplasmic membrane initially forms a cup-like structure through invagination, allowing extracellular components to enter the cell via endocytosis along with cell surface proteins to create early endosomes (ESE) [24]. These ESEs can exchange substances with the endoplasmic reticulum and the trans-Golgi tubular network, leading to the formation of late endosomes (LSE) [25–27]. LSE membranes undergo invagination and envelop intracellular specialized proteins, nucleic acids, and other substances to generate vesicles known as ILV [28,29]. The content within these ILVs represents the cargo intended for transport by future exosomes. Depending on the extent of invagination, this process can result in ILVs of varying inclusions and sizes, ultimately giving rise to multivesicular vesicles (MVBs). MVBs may undergo degradation by merging with autophagosomes or lysosomes, with the resulting breakdown products being recyclable by the cell [13]. Additionally, MVBs can be transported to the plasma membrane through the cell's cytoskeleton and microtubule network [14], ultimately being secreted as EVs ranging in diameter from 30 to 150 nm.

EVs share common marker proteins, including CD63, CD81, and CD9 [30,31], involved in exosomal transport, as well as plasma membrane proteins like flotillin [32], and proteins like Alix and TSG101 incorporated during MVB processing [5]. However, due to the diverse origins and environments of the cells they are derived from, EVs exhibit complexity and heterogeneity in their composition. Compared with parental cells , EVs can accumulate nucleic acids and proteins during processing, which are safeguarded by phospholipid bilayers for stability [33,34]. As a result, EVs are enriched in RNA and proteins [35,36], making them valuable sources of information for liquid biopsies and essential effectors for cellular regulatory functions [37,38] (Fig. 1).

### 2.3. Mechanism of MV formation

Compared to exosomes, the molecular mechanisms of MV release remain relatively understudied. MV biogenesis occurs through direct outward blistering and extrusion of the plasma membrane, leading to the release of nascent microvesicles into the extracellular space. This shedding process involves molecular redistribution of the plasma membrane, with a key role played by the small GTPase protein ADP-ribosylation factor 6 (ARF6). Activation of ARF6 triggers myosin light chain phosphorylation and actinomyosin contraction, facilitating the release of microvesicles [39]. Furthermore, endosomal sorting complex required for transport (ESCRT) components, ras homolog gene family member A (RhoA), and intracellular calcium levels also play important roles in regulating MV release [40–42].

### 2.4. Mechanisms of apoptotic vesicle formation

Apoptotic vesicles are formed by cells during apoptosis, characterized by the wrinkling and invagination of the cell membrane. These vesicles contain DNA material and organelles, and their formation involves stages such as nuclear chromatin condensation, nuclear division with micronuclei, membrane vesiculation, and the splitting of cellular contents into apoptotic vesicles [17,43]. Once considered as cell fragments, recent studies have highlighted the important role of apoptotic vesicles in intercellular communication related to human health and various diseases [44]. This newfound understanding has opened up possibilities for utilizing apoptotic vesicles in disease development and drug delivery.

Exosomes originate from the endosomal pathway and are secreted by MVB fused to the plasma membrane. Microvesicles are produced by direct outward budding from the plasma membrane. Apoptotic vesicles are vesicles released during apoptotic cell death (left). Exosomes have a lipid bilayer membrane structure and contain important information such as multiple proteins, lipids and RNA from the mother cell (right). ER, endoplasmic reticulum; MVB, multivesicular body.

### 2.5. EVs derived from normal hepatocytes

Hepatocytes play a central role in the body's response to stress and the metabolism of endogenous as well as exogenous substances. While limited studies have focused on the biological properties of EVs from normal hepatocytes, proteomic analysis suggests that they may be involved in various processes typically associated with hepatocytes, such as fat and glucose metabolism [45].

Hepatocytes communicate with each other through EVs. Studies have shown that primary mouse Hep-EVs directly fuse with target

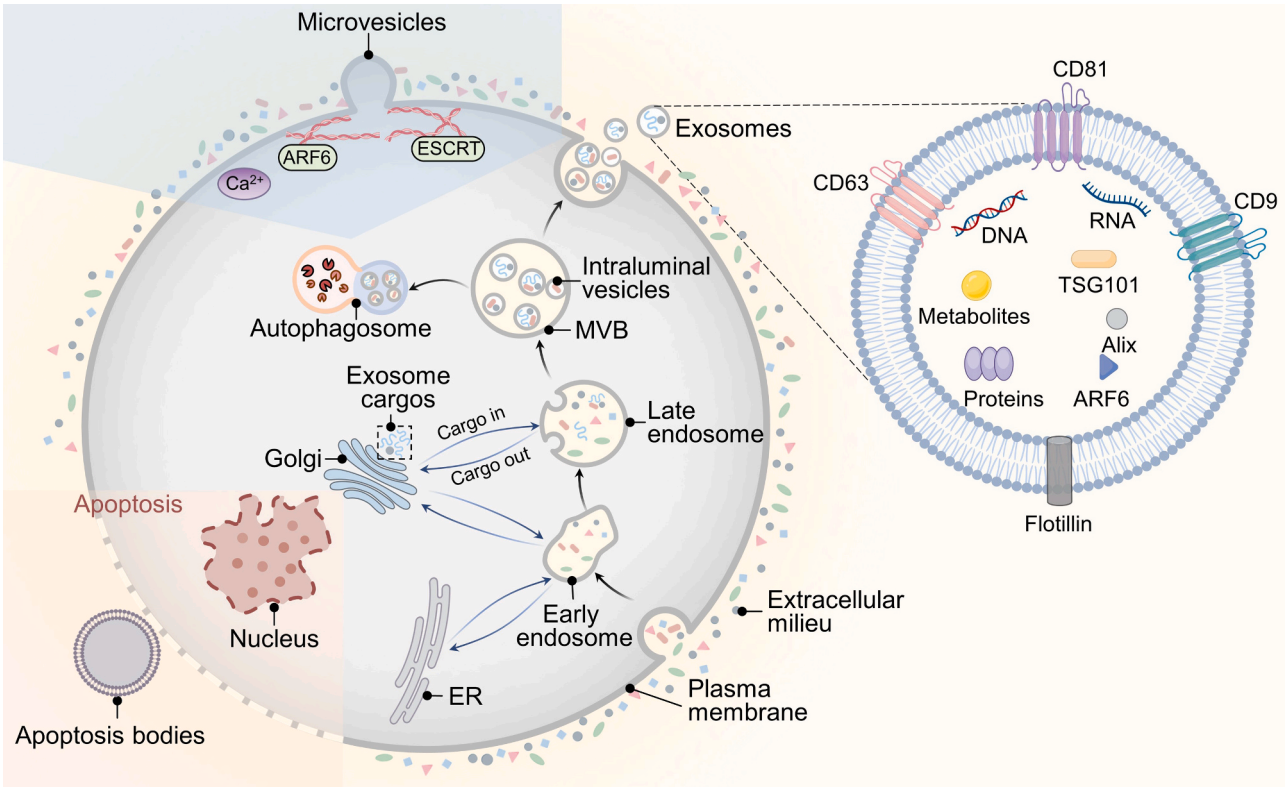


**Fig. 1.** Mechanism of exosome formation and composition.

hepatocytes, transferring neutral ceramidase and sphingosine kinase 2 (SK2). This transfer leads to an increase in sphingosine-1-phosphate (S1P) synthesis within the recipient cells, ultimately promoting hepatocyte proliferation. In contrast, EVs from Kupffer cells or liver sinusoidal endothelial cells do not have the same proliferative effect. This highlights the unique role of Hep-EVs in restoring liver homeostasis following injury, dysfunction, or disease [46]. Despite their therapeutic potential, the limited natural production of EVs poses a challenge for large-scale applications. Recent research has demonstrated a method of generating Exosome-Mimetic Nanovesicles by continuously extruding cells through polycarbonate membranes. This innovative approach yields more than 100 times the quantity of natural EVs, making Exosome-Mimetic Nanovesicles derived from primary hepatocytes a promising tool for advancing regenerative medicine [47].

Hepatocytes communicate with neighboring nonparenchymal cells through EVs. These EVs derived from hepatocytes play a regulatory role in HSCs by modulating the TGF-β/Smad signaling pathway, promoting the synthesis and activation of nuclear factor erythroid-2-related factor 2 (Nrf2) in liver tissue, and increasing the expression of protective factors to combat oxidative damage [48]. The miRNAs abundant in Hep-EVs, particularly miR-423–5p, are crucial in maintaining HSCs in a quiescent state by reducing the levels of HSCs differentiation markers. Additionally, Hep-EVs treated with salidroside are rich in miR-146a-5p, which inhibits HSCs activation and liver fibrosis by targeting the epithelial-to-mesenchymal transition processes [49]. These findings suggest that Hep-EVs hold promise as a therapeutic approach for liver fibrosis [50]. Furthermore, research by Liu et al. demonstrated that Hep-EVs can also inhibit the secretion of inflammation-related cytokines, and its inhibitory effect on the expression of inflammation-related genes tumor necrosis factor alpha (TNF-α) and IL-6 in macrophages is even stronger than that of mesenchymal stem cell EVs [51].

Hep-EVs have been shown to have an impact beyond the liver. For example, miR-203a-3p has been reported to reduce the invasion rate of colorectal cancer (CRC) cells by inhibiting Src expression, increasing E-cadherin levels, and promoting mesenchymal-epithelial transition in CRC cells [52]. Furthermore, the liver-brain axis is significant in the body, with the selenoprotein p (SELENOP) secreted by the liver providing selenium for other selenoproteins synthesis. SELENOP can be transported by Hep-EVs across the blood-brain barrier to neural cells, protecting them from cell death induced by β-amyloid protein [53]. The liver-bone axis is a recent theory [54], indicating that leucine-rich alpha-2-glycoprotein 1(LRG1)-containing Hep-EVs can inhibit osteoclast differentiation by inhibiting NF-κB p65 into the nucleus and transcribing NFATC1. This is critical for maintaining bone homeostasis and may be a potential target for the treatment of primary osteoporosis [55]. In conclusion, EVs from normal hepatocytes play a crucial role in preserving normal physiological functions and metabolic stability in the human body (Fig. 2).

In normal liver, EVs released from the liver carries biomolecules to adjacent hepatocytes, hepatic stellate cells, macrophages, and extrahepatic colon cancer cells, neuronal cells, and osteoclasts. This maintains the normal physiological function of the organism. SELENOP, selenoprotein p; SK2, sphingosine kinase 2; S1P, sphingosine-1-phosphate.

### 2.6. *EVs derived from lipotoxic hepatocyte (LTH-EVs)*

Lipotoxicity is a term commonly used to describe the biological processes that result in cell damage and death due to free fatty acids (FFA) and their metabolites [56]. Lipotoxic hepatocytes refer to the excessive accumulation of FFA in hepatocytes, leading to the production of toxic lipid metabolites and the onset of NAFLD [57]. Key factors that contribute to lipotoxic damage in hepatocytes include free cholesterol, FFA, and the derivative ceramide [58,59]. Research has demonstrated that a combination of low-concentration oleic acid (OA) and palmitic acid (PA) in a 2:1 ratio over 2 days can effectively establish a fatty liver cell model, resulting in oxidative stress and mitochondrial damage in hepatocytes [60]. Currently, the use of OA and PA in combination or PA alone is widespread in cellular models for studying NAFLD. Oil Red O

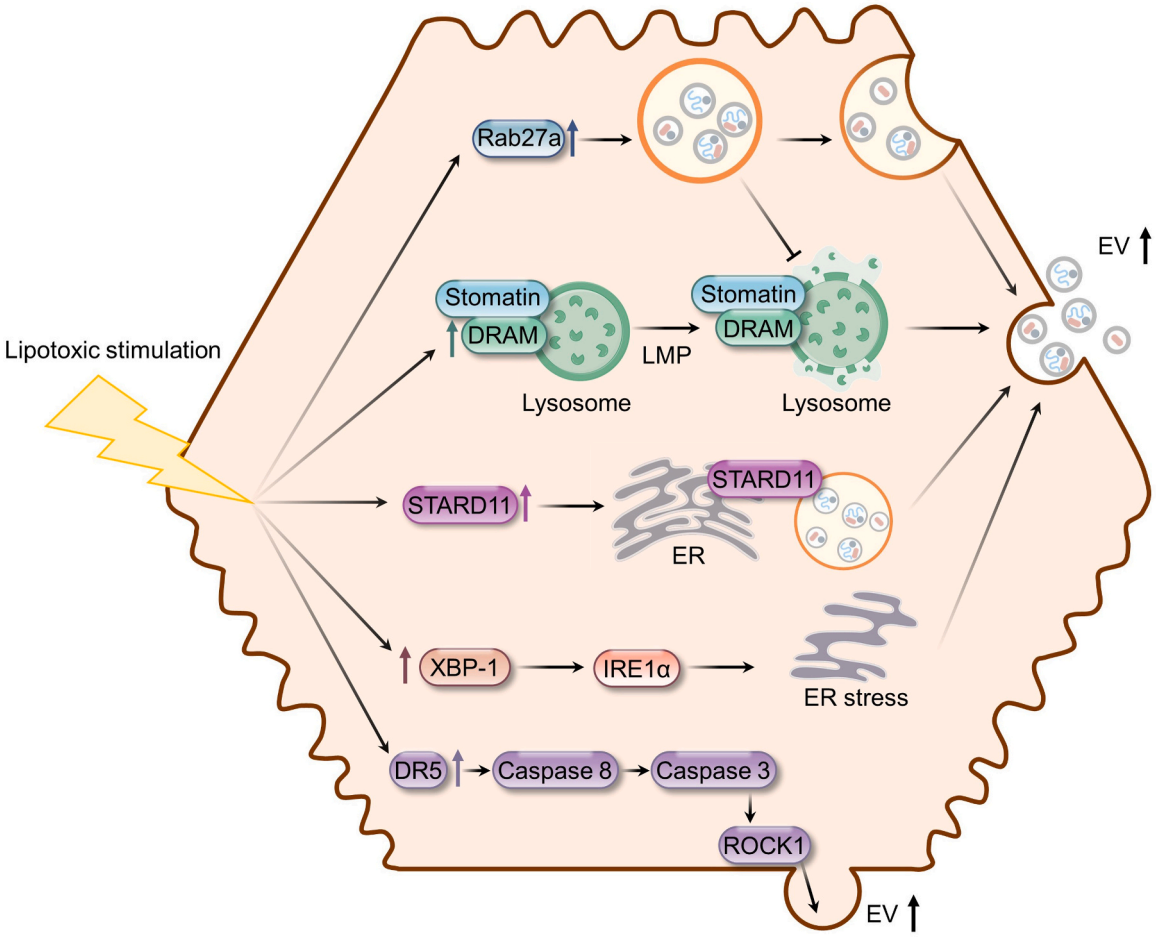


**Fig. 2.** Role of normal Hep-EVs in the organism.

staining, along with measurements of triglycerides, malondialdehyde, and reactive oxygen species, demonstrated an increase in lipid accumulation and oxidative stress in hepatocytes. Furthermore, cell viability assessments indicated a reduction in hepatocyte proliferation [61,62]. Collectively, these indicators confirm the successful establishment of the NAFLD model. Lipotoxic hepatocytes play a crucial role in triggering hepatic inflammation and apoptosis. LTH-EVs various biomolecules that contribute to the pathogenesis and progression of NAFLD [63]. Therefore, this study delves into the changes in EVs numbers in lipotoxic hepatocytes and their impact on different cell types.

### *2.7. Lipotoxicity induces increased release of EVs derived from hepatocytes*

Numerous studies have demonstrated that lipotoxicity can lead to an increase in LTH-EVs [64]. However, the specific mechanism by which lipotoxicity promotes EVs release has been relatively understudied. It is widely recognized that EVs release in cells is intricately linked to lysosomal function. Povero et al. found that lipid-induced damage-regulated autophagy modulator in hepatocytes can interact with stomatin, facilitating the translocation of stomatin to lysosomes. This interaction induces lysosomal membrane permeabilization (LMP), which in turn inhibits lysosomal degradation of MVBs, leading to an increased fusion of MVBs with the plasma membrane and subsequent EVs release [65]. Furthermore, as endosomes progress from early to late stages to form MVBs, they closely interact with the endoplasmic reticulum. Another study revealed that PA enhances the colocalization of the ceramide transporter StAR-related lipid transfer domain 11 (STARD11) with MVBs and the endoplasmic reticulum, facilitating EVs release [66]. Additionally, Kakazu et al. demonstrated that palmitic acid induces endoplasmic reticulum stress through the inositol requiring enzyme 1α (IRE1α) / X-box binding protein-1 pathway, thereby stimulating EVs release in hepatocytes [67]. Moreover, Rab27a plays a crucial role in mediating the fusion of MVBs with the cell membrane and serves as a key regulatory factor in EVs release. Lipotoxicity triggers an upregulation of Rab27a expression in hepatocytes, consequently promoting EVs production [68]. Notably, Petra et al. found that hepatocytes treated with PA released a larger volume of EVs compared to untreated hepatocytes [69]. They suggest that EVs induced by lipotoxicity may originate from microvesicles in the plasma membrane rather than traditional exosomes. This increase in microvesicle release is attributed to rho-associated coiled-coil containing protein kinase 1 (ROCK1) promoting plasma membrane blebbing and shedding of microvesicles [70]. The differences in EVs number and composition between lipotoxic and normal hepatocytes significantly impact their physiological functions (Fig. 3).

Molecular mechanism of increased extracellular vesicle release in hepatocytes after lipotoxic stimulation. DRAM, Damage-regulated autophagy modulator; STARD11, StAR-related lipid transfer domain 11; LMP, Lysosome membrane permeates; XBP-1, IRE1α, inositol requiring enzyme 1α; ER, endoplasmic reticulum; ROCK1, coiled-coil-containing protein kinase 1.

### *2.8. LTH-EVs promote HSCs activation*

Non-alcoholic steatohepatitis (NASH) is a crucial stage in the advancement of NAFLD [71]. If left untreated, NASH can progress to fibrosis [72], significantly increasing the patient's risk for adverse liver

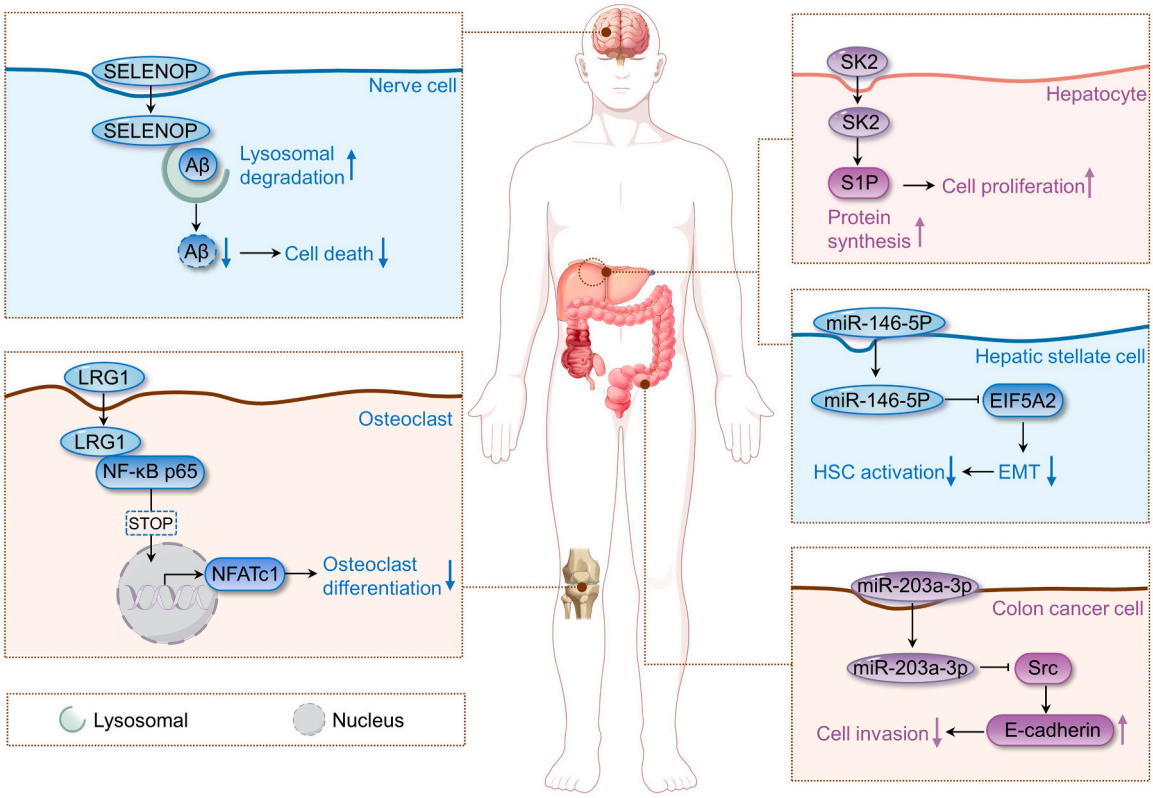


**Fig. 3.** The development mechanism of extracellular vesicles in nonalcoholic fatty liver.

injury outcomes. The sustained activation of HSCs plays a crucial role in the development and progression of liver fibrosis [73]. Normally, HSCs are in quiescent state, involved in vitamin A metabolism and fat storage. However, under pathological conditions, triggered by physical, chemical, viral infections, and other biological factors, HSCs become activated, proliferate, transform into myofibroblasts, express α-SMA, and synthesize extracellular matrix [74].

Several studies have demonstrated that EVs from PA-treated hepatocytes can activate HSCs [75]. Specifically, PA stimulation leads to the release of EVs enriched with miR-107, which are then transferred to HSCs. The transferred miR-107 activates the Wnt signaling pathway by targeting dickkopf related protein 1 (DKK1), ultimately inducing HSCs activation [76]. Moreover, research by Luo et al. revealed that LTH-EVs contain elevated levels of miR-1297, which can be transferred to HSCs and promote their activation through the PTEN/PI3K/AKT pathway [77]. Additionally, an increase in miR-27a, a miRNA associated with mitochondrial function, was observed in LTH-EVs. Higher levels of miR-27a were found to correlate with increased liver fibrosis severity, as it inhibits mitophagy by downregulating PINK1 expression, leading to HSCS transformation into fibroblasts and promoting liver fibrosis [78]. The activation of HSCs impacts mitochondrial homeostasis [79], with mitophagy inhibition preventing HSCS apoptosis [80]. LTH-EV-derived LIMA1 interacts with PINK1 in HSCs and promotes protein degradation of PINK1, thereby inhibiting mitophagy and promoting hepatic stellate cell activation [81]. Moreover, LTH-EVs with increased miR-128–3p levels inhibit peroxisome proliferator-activated receptor-γ (PPAR-γ) expression in HSCs, leading to a shift from a quiescent to an activated phenotype [82]. LTH-EV releases ferritin to hepatic HSCs. The accumulation of ferritin results in excessive production of reactive oxygen species (ROS), which subsequently promotes fibrosis in HSCs [83]. Interestingly, LTH-EVs exhibit higher intracellular uptake efficiency in activated HSCs compared to those from untreated hepatocytes [69]. Overall, lipotoxicity-induced changes in EVs release, content, and tropism from hepatocytes can enhance HSCs activation.

### 2.9. LTH-EVs promote liver inflammation

NAFLD is primarily a metabolic disorder, but it also involves inflammatory processes driven by various immune cells, particularly during the progression to NASH. A key characteristic of NASH is the presence of macrophage-mediated sterile inflammation [74]. Macrophages can polarize into two distinct phenotypes-classically activated (M1 type) macrophages that release inflammatory cytokines and alternatively activated (M2 type) macrophages that produce anti-inflammatory cytokines [84]. A study discovered that LTH-EVs containing tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). These EVs then activate macrophages via the Death receptor 5 (DR5) signaling pathway in a receptor interacting protein 1 (RIP1)-dependent fashion [70]. Furthermore, LTH-EVs containing retinol-binding protein 4 (RBP4) activate the NADPH oxidase 2 (NOX2) / nuclear factor κb (NF-κB) pathway, causing a shift in macrophage polarization from M2 to M1 and resulting in the secretion of pro-inflammatory cytokines that contribute to liver lipid accumulation and inflammation [85]. Additionally, LTH-EVs transport miR-192–5p, which regulates the Rictor/Akt/FoxO1 signaling pathway and plays a crucial role in activating pro-inflammatory macrophages and driving the progression of NAFLD [86].

It is worth noting that macrophages are located in the hepatic sinusoidal cavity and often attach to endothelial cells through their pseudopodia or extend into the perisinusoidal space through endothelial fenestra and intercellular spaces. On the one hand, the Malhi team reported that LTH-EVs activates macrophage chemotaxis by transferring ceramide to macrophages, providing a substrate for macrophages to produce S1P [67]. This team subsequently reported that LTH-EVs was also rich in S1P and recruited macrophages expressing S1P1 receptor to the liver [87]. Similarly, lipotoxic hepatocytes release a potent chemokine CXC motif ligand 10-bearing (CXCL10) that induces macrophage chemotaxis toward the liver [88]. On the other hand, LTH-EVs can accelerate their movement to macrophages. It has been reported that

matrix metalloproteinase 2 (MMP2) is present on the outside of EVs released from lipotoxic hepatocytes. The presence of MMP2 enables EVs to migrate through the extracellular matrix by degrading the extracellular mesh composed of collagen, thereby ensuring rapid movement of EVs in the matrix and promoting uptake by macrophages [89]. Therefore, LTH-EVs provide insights into the mechanisms of hepatic macrophage activation and chemotaxis in NASH.

### 2.10. Effects of LTH-EVs on liver sinusoidal endothelial cells (LSECs)

LSECs are the predominant cell type among liver non-parenchymal cells and a key component of liver sinusoids. Normally, LSECs possess fenestra and lack a structured basement membrane, which supports their endocytic function and helps in regulating hepatic vascular tone and portal vein pressure. However, under pathological conditions, LSECs undergo capillarization, leading to angiogenesis and vasoconstriction. Povero et al. highlighted the significance of LTH-EVs in promoting angiogenesis. These EVs carry vanin-1 (VNN1), an enzyme involved in cell adhesion and migration, and are internalized by endothelial cells. Notably, VNN1-deficient hepatocyte EVs fail to induce significant angiogenesis [90]. However, the connection and role between LTH-EVs and LSECs have been rarely studied, which may be related to the reduced EVs uptake by LSECs in hyperlipidemia [91]. In summary, LTH-EVs in NAFLD can drive capillarization of LSECs, exacerbating the advancement of NAFLD (Fig. 4).

Some of the biomolecules loaded by EV are up-regulated and act on hepatic stellate cells, macrophages and liver sinusoidal endothelial cells in the liver to promote hepatic inflammation and fibrosis levels. AKT, Protein Kinase B; CXCL10, CXC motif ligand 10-bearing; DKK1, Dickkopf Related Protein 1; FOXO1, Forkhead Box Protein O1; MMP2, matrix metalloproteinase 2; NOX2, NADPH oxidase 2; NF-κB, nuclear factor κb; PI3K, phosphatidylinositol 3-kinase; PINK1, PTEN induced putative kinase 1; PPARδ, peroxisome proliferator-activated receptor δ; PTEN, Phosphatase and tensin homolog; RBP4, retinol-binding protein 4; Rictor, rapamycin-insensitive companion of mTOR; RIP1, Receptor Interacting Protein 1; S1P, sphingosine-1-phosphate; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; VNN1, vanin-1.

### 2.11. Effects of LTH-EVs on pancreatic islets

NAFLD is widely present in diabetic patients, and more than half of diabetic patients have NAFLD [92]. Insulin resistance plays a key role in the development of NAFLD-related dyslipidemia [93]. EVs in mouse hepatocytes induced by high-fat diet highly express miR-7218–5p and induce islet β-cell proliferation by targeting the CD74 gene, which may be a pathway for compensatory proliferation of islet β-cells under conditions of obesity and insulin resistance [94]. Ji et al. further studied the regulatory effect of LTH-EVs on pancreatic islets in the early and late stages of obesity. The high expression of miR-3075 in the EVs of hepatocytes fed HFD for 4 weeks resulted in a decrease in fatty acid 2-hydroxylase (FA2H) and an increase in glucose uptake. This is the body's compensatory response to the early stages of caloric excess to protect the

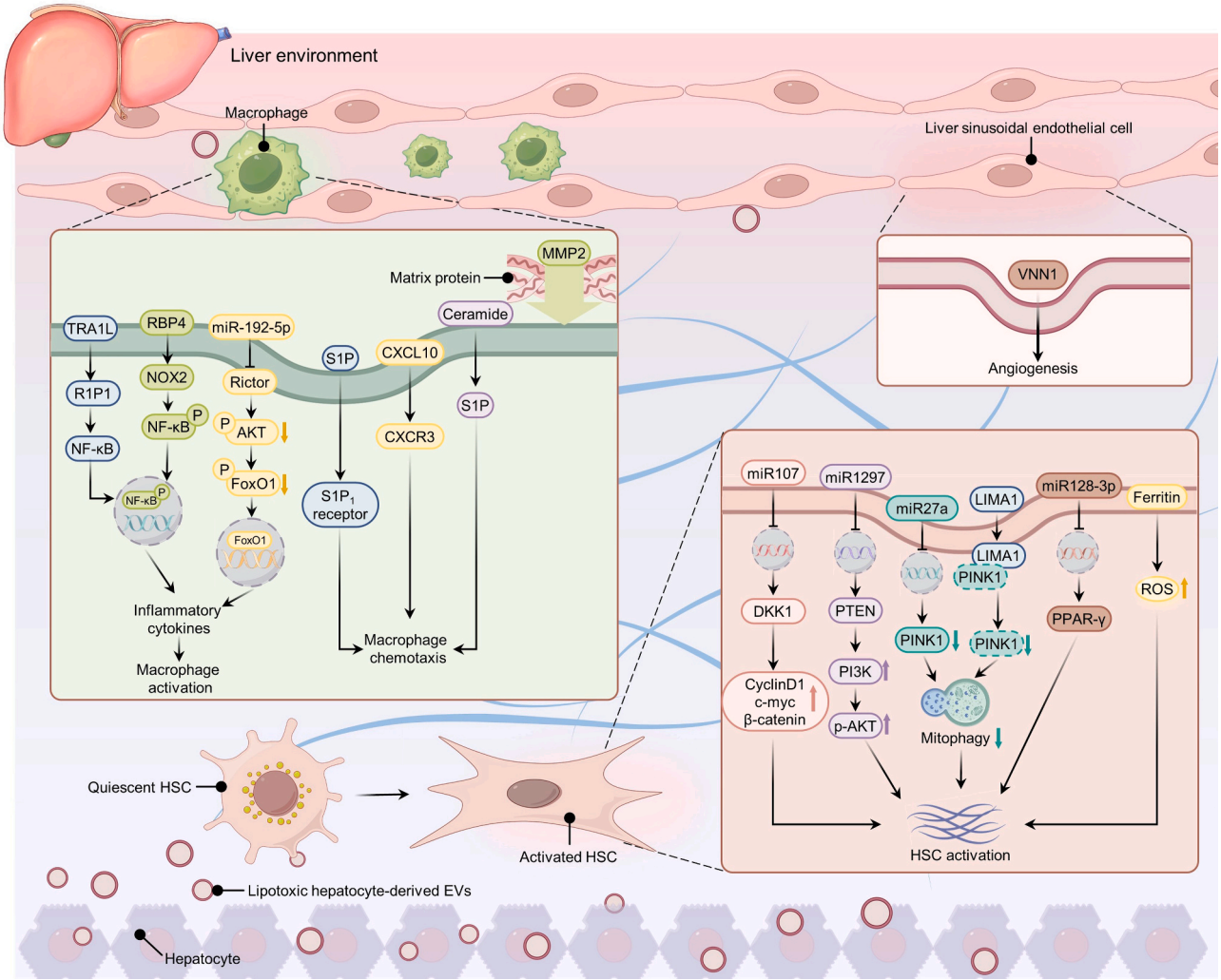


**Fig. 4.** The role of LTH-EVs in liver.

body from insulin resistance caused by chronic obesity. However, as obesity develops into a chronic state, hepatocyte EVs at HFD 16 W no longer contain high levels of miR-3075, but express high levels of miR-434–3p, which can promote macrophages to M1. The state is polarized and activates macrophage pro-inflammatory responses to promote insulin resistance [95]. In conclusion, LTH-EVs provide different signals to regulate the systemic insulin-sensitive state. In the early stages of obesity, hepatocytes secrete EVs that promote the insulin-sensitive state. As obesity develops into a chronic state, hepatocytes secrete EVs that promote insulin resistance and glucose tolerance.

### 2.12. Effects of LTH-EVs on cardiovascular

NAFLD and cardiovascular disease (CVD) are both manifestations of the metabolic syndrome, and they are related to each other through multiple pathophysiological substrates [96]. Hepatocyte EVs can easily cross fenestrations in the sinusoidal endothelium and carry hepatocyte-specific contents into the bloodstream [97]. MiR-1 derived from LTH-EVs promotes endothelial inflammation and atherosclerosis by reducing kruppel-like factor 4 (KLF4) expression and activating NF-κB [98]. Inflammation of endothelial function is an early stage in the development of atherosclerosis, and the inflammatory response causes macrophages to take up cholesterol and fatty acids and form foam cells. Cholesterol-rich foam cells are a hallmark of the progression of atherosclerosis. Chen et al. explored whether LTH-EVs would further affect foam cell formation. They pointed out that lipotoxic hepatocyte-derived EVs enriched in miR-30a-3p inhibited reverse cholesterol transport in macrophages by targeting the ATP-bindingcassettetransporterA1 (ABCA1) pathway, leading to foam cell formation and atherosclerosis progression [99]. Not only that, NAFLD patients are more prone to coronary microvascular dysfunction, and LTH-EVs enriched novel microRNA-7 is the cause of Nucleotide-binding domain leucine-rich repeat pyrin domain containing 3 (NLRP3) inflammasome-dependent endothelial barrier dysfunction. miR-7 directly targets lysosomal associated membrane protein 1 (LAMP1) and LMP, thereby inducing cathepsin B-dependent NLRP3 inflammasome activation and microvascular endothelial hyperpermeability [100]. CVD is one of the major causes of death in NAFLD, and LTH-EVs are key mediators of long-range communication between the liver and cardiovascular system, suggesting a mechanistic link between NAFLD and atherosclerotic CVD.

### 2.13. Effects of LTH-EVs on tumor metastasis

There is direct blood circulation through the portal vein between the intestine and the liver, which is also the most common site of metastasis of CRC. Fatty liver will increase the incidence of colorectal cancer liver metastases (CRLM) and the local recurrence rate after resection of CRLM [101]. Studies have shown that NAFLD significantly increases the number and size of CRC liver metastases. In the presence of NAFLD, tumor-associated macrophages (TAMs), M2 macrophages, significantly accumulate in tumors, thereby promoting the growth of metastatic liver tumors [102]. Wang et al. further demonstrated the role of EV in CRLM. The expression of miR25, miR92, and miR103 in fatty liver EVs is upregulated and absorbed by colon cancer cells. These miRNAs inhibit large tumor suppressor homolog 2 (LATS2) and thereby enhance yes-associated protein (YAP) activity. YAP promotes cancer cell growth and the immunosuppressive microenvironment of M2 macrophage infiltration by increasing the expression of cysteine rich protein angiogenic inducer 61 (CYR61) [68]. In short, fatty liver-derived EVs create a liver microenvironment that precedes CRLM.

In summary, during the development of NAFLD, EVs regulate signal transduction and material transfer between hepatocytes, HSCs and macrophages, and are transported to extrahepatic organs to influence cardiovascular disease, insulin resistance, and tumor metastasis (Fig. 5).

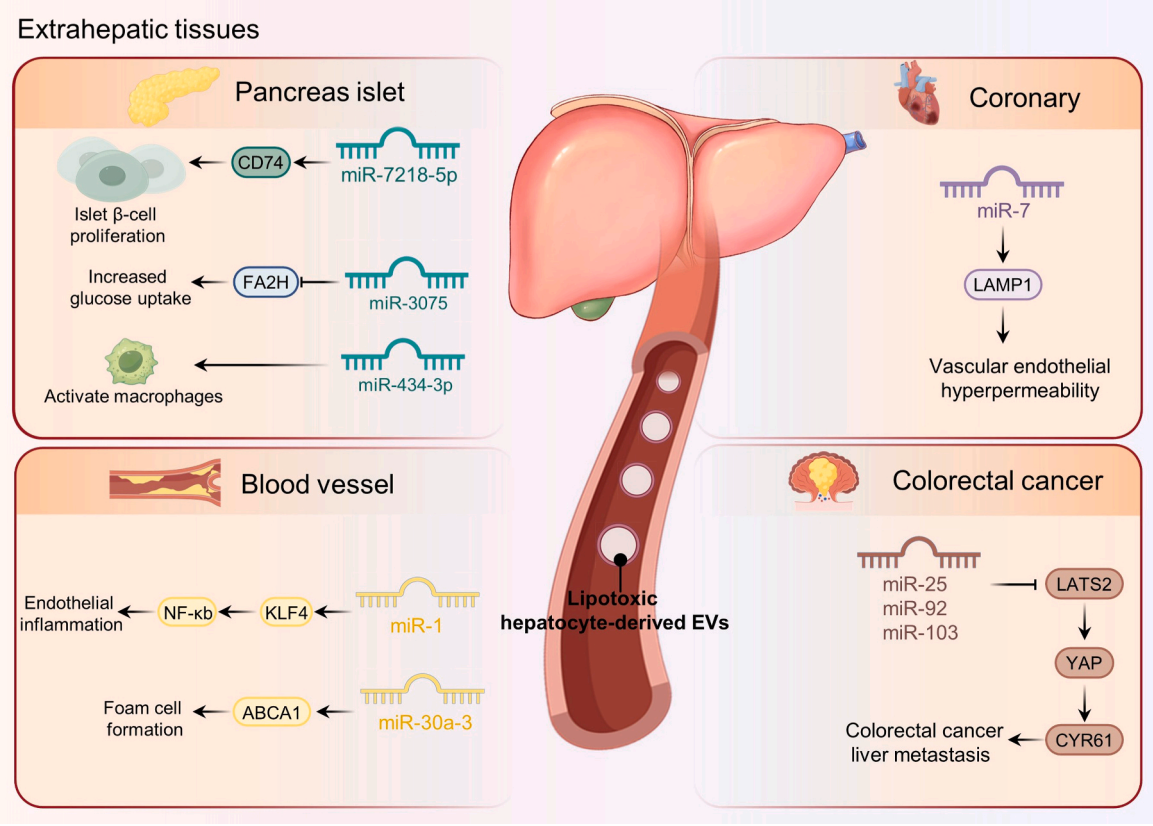


**Fig. 5.** The role of LTH-EVs in the extrahepatic environment.

LTH-EVs can be transported over long distances through the bloodstream to extrahepatic sites such as pancreatic islets, blood vessels, and the colorectum, and plays an important role in the communication between the liver and other organs. FA2H, fatty acid 2-hydroxylase; LAMP1, lysosomal associated membrane protein 1; KLF4, kruppel-like factor 4; ABCA1, ATP-bindingcassettetransporterA1; LATS2, large tumor suppressor homolog 2; YAP, yes-associated protein.

## 3. EVs derived from hepatocytes in other states

In addition to NAFLD, liver cells can experience various forms of damage, including alcoholic liver disease (ALD), viral hepatitis, drug-induced liver injury, and liver cancer. These conditions also lead to an increased release of Hep-EVs. For instance, research conducted by Bala et al. demonstrated that alcohol exposure stimulates the release of EVs by upregulating the mRNA expression of Rab27b [103]. Hepatocellular carcinoma (HCC) ranks among the most prevalent tumors globally, often arising in the context of cirrhosis [104]. On one hand, HCC is a highly vascularized tumor. HCC-derived EVs promote angiogenesis, ensuring the provision of adequate oxygen and nutrients to the tumor, thereby playing a crucial role in tumor growth and metastasis [105]. Furthermore, HCC-derived EVs modulate the tumor microenvironment, enhancing both the development and metastasis of HCC. Hepatitis B virus (HBV) and hepatitis C virus (HCV) are two significant pathogens that can lead to severe viral hepatitis. In this context, virus-infected hepatocytes release EVs containing viral genomes, protein components, or even complete virions, which can facilitate the spread of infection among hepatocytes [106]. Additionally, EVs derived from HBV and HCV-infected hepatocytes play a critical role in evading the host's immune defenses. Table 1 summarizes these reports.

**Table 1**
EVs derived from hepatocytes in other states.

| Disease | Content | Mechanism | Ref. |
|---|---|---|---|
| ALD | Hsp90 | Activate macrophages | [107] |
| | PKM2 | Activate macrophages | [108] |
| HCC | miR−1290 | Enhanced angiogenesis | [109] |
| | miR−210 | Enhanced angiogenesis | [110] |
| | LOXL4 | Enhanced angiogenesis | [111] |
| | miR−200b−3p | Proliferation and activation of macrophages | [112] |
| | miR−21–5p | Polarization of TAM | [113] |
| | miR−23a | Up-regulated expression of PD-L1 in macrophages | [114] |
| | miR−574–5p | Regulate osteoclast differentiation | [115] |
| Virus | HBV nucleic acids | Impaired NK cell function | [116] |
| | HCV RNA | Regulate T follicular cell differentiation | [117] |
| | miR−506–3p | Activate hepatic stellate cells | [118] |
| | miR−222 | Activate hepatic stellate cells | [119] |
| Hepatotoxicants | Arginase | induce pulmonary endothelial dysfunction | [120] |
| | miR−194 | Promote angiogenesis | [121] |
| | miR−222–5p | Increase the proliferation of HCC | [122] |
| | MALAT1 | Activate hepatic stellate cells | [123] |
| | H2AFJ | Activate hepatic stellate cells | [124] |
| | MASP1 | Activate hepatic stellate cells | [125] |
| | Mitochondria-cargo | Activate hepatic stellate cells | [126] |
| | CYTOR | Activate hepatic stellate cells | [127] |
| Ischemia reperfusion | miR−122–5p | Activate macrophages | [128] |

Abbreviation: ALD, alcoholic liver disease; Hsp90, heat shock protein 90; PKM2, pyruvate kinase M2; LOXL4, lysyl Oxidase Like Protein 4; H2AFJ, H2A Histone Family Member; MASP1, mannose associated serine protease 1; CYTOR, cytoskeleton regulator RNA.

## 4. EVs as biomarkers

Invasive liver biopsy is currently considered the gold standard for diagnosing liver disease, but its expense and invasiveness make it unsuitable for screening purposes. The early symptoms of liver disease can be subtle and challenging to identify, highlighting the importance of early intervention to halt disease progression. As a result, there is a pressing clinical demand for novel, dependable, and non-invasive diagnostic approaches. EVs containing proteins, lipids, and nucleic acids serve as messengers between cells, with an increase in EV release from damaged hepatocytes and the overexpression of certain proteins or nucleic acids in these cells. Consequently, EVs are increasingly recognized as promising biomarkers for a range of liver diseases. Table 2 summarizes these reports.

### 4.1. EVs as biomarkers for NAFLD

Liver biopsy is widely recognized as the gold standard for diagnosing and staging NAFLD. However, there is a need for easily applicable non-invasive biomarkers to enable early diagnosis [129]. As NASH progresses, circulating EVs remain elevated, originating from various cells and organs, with approximately 20 % expressing hepatocyte-specific markers [130]. EVs derived from hepatocytes increase in the early stages of NAFLD but decrease as the disease progresses to cirrhosis [131]. Newman et al. conducted an analysis on serum EVs from NAFLD patients, revealing significantly higher levels of miR-122, miR-192, and miR-128–3p in liver-specific asialoglycoprotein receptor 1 (ASGPR1)--positive EVs compared to healthy controls, suggesting a correlation between miRNA levels and NAFLD severity [132]. Nakao et al. utilized asialoglycoprotein receptor 2 (ASGR2) and cytochrome P450 2E1 (CYP2E1) as markers for Hep-EVs, noting reduced levels of these markers in NAFLD patients post-bariatric surgery [133]. Additionally, a separate study demonstrated elevated levels of mitochondrial DNA and intact mitochondria in the plasma of mice and NASH patients, which could activate toll like receptor 9 (TLR9) [134].

### 4.2. EVs as biomarkers for ALD

MiRNA plays a crucial role in diagnosing ALD pathogenesis. Previous research indicates that miRNA-192, miRNA-122, and miRNA-30a are effective in identifying alcoholic liver injury [135]. Momen et al. observed an increase in hepatocyte-specific miRNA-122 levels in exosomes from the serum of individuals post-alcohol abuse, leading to the indirect production of pro-inflammatory factors in macrophages [136]. Eguchi et al. discovered that let7f, miR-29a, and miR-340 were elevated in blood EVs of alcohol-injured mice, offering a potential diagnostic approach for ALD [137]. Moreover, alcohol-induced hepatocyte EVs are enriched in mtDNA, likely due to mitochondrial damage in AH liver [138]. The proteins abundant in EVs can serve as diagnostic markers for ALD, with CYP enzymes in EVs remaining metabolically active. CYP2E1-containing EVs are secreted from hepatocytes and accumulate in the plasma of alcohol-exposed mice and patients, indicating their potential as biomarkers of alcohol injury [139]. Furthermore, caspase activation in alcohol-stimulated hepatocytes leads to the release of CD40L-rich EVs, with elevated levels observed in the serum of patients with alcoholic hepatitis [140].

### 4.3. EVs as biomarkers for HCC

Research on EVs diagnosis of liver diseases has predominantly centered on HCC. Zhou et al. discovered that the expression of miRNA-21 in serum EVs of HCC patients was significantly elevated compared to healthy controls, with high levels in HCC tissues predicting unfavorable outcomes [141]. Another study revealed higher levels of miR-18a, miR-221, miR-222, and miR-224 in serum EVs of HCC patients compared to those with hepatitis B infection, while miR-101, miR-106b,

**Table 2**
Hepatocellular derived or circulating extracellular vesicles as biomarkers for the diagnosis of liver diseases.

| Disease | Source | Content | Expression | Effect | Ref. |
|---|---|---|---|---|---|
| NAFLD | Hepatocytes | ASGR1, SLC27A5 | Up | N/A | [129] |
| | Hepatocytes | miR−122, miR−192, miR−128–3p | Up | N/A | [130] |
| | Hepatocytes | ASGR2, CYP2E1 | Down in weight loss surgery in NAFLD patients | N/A | [131] |
| | Hepatocytes | mtDNA | Up | Activate TLR9 | [132] |
| ALD | Serum | miRNA−192, miRNA−30a | Up | Regulated TGFβ/Smad signaling and Jak2/Arhgef1 signaling pathways | [133] |
| | Hepatocytes | miR−122 | Up | Increased levels of pro-inflammatory cytokines | [134] |
| | Serum | let-7f, miR−29a, miR−340 | Up | N/A | [135] |
| | Hepatocytes | mtDNA | Up | Upregulated IL−1β and IL−17 production by macrophages | [136] |
| | Hepatocytes | CD40L | Up | Promoted macrophage activation | [138] |
| HCC | Hepatocytes | miRNA−21 | Up | Converted normal HSCs into cancer-associated fibroblasts | [139] |
| | Serum | miR−18a, miR−221, miR−222, miR−224 | Up | N/A | [140] |
| | Serum | miR−101, miR−106b, miR−122, miR−195 | Down | N/A | [140] |
| | Serum | circPTGR1 | Up | Enhanced cell migration and invasion potential | [141] |
| | Serum | miR−638 | Down | Affected cell proliferation | [142] |
| Viral hepatitis | Hepatocytes | miR−19a | Up | Modulated the SOCS-STAT3 axis to activate HSCs | [143] |
| | Plasma | miR204–5p, miR181a−5p, miR143–3p, miR93–5p, miR122–5p | Down | N/A | [144] |
| | Hepatocytes | miR−122 | Up | Formed miR−122-Ago2-HSP90 complexes | [145] |

Abbreviation: Up, upregulated; down: downregulated; N/A, not applicable; HCC, hepatocellular carcinoma; ALD, alcoholic liver disease; NAFLD, nonalcoholic fatty liver disease; TLR9, Toll-like receptor 9; TGF-β, transforming growth factor-β; Smad: small mother against decapentaplegic; Jak2, Janus kinase2; Arhgef1, Rho guanine nucleotide exchange factor 1; SOCS, suppressor of cytokine signaling; STAT3, signal Transducer and Activator of Transcription 3; CYP2E1, cytochromeP450 family2 subfamily E polypeptide1; mtDNA, mitochondrial DNA; CD40L, CD40 antigen ligand.

miR-122, and miR-195 levels were lower. However, these microRNA levels did not show correlation with tumor characteristics such as stage, invasion, or differentiation [142]. Cells with greater invasive potential can transfer circPTGR1 to less invasive cells via EVs, enhancing migration and invasion capabilities. circPTGR1 is upregulated in serum EVs of HCC patients and correlates with clinical stage and prognosis [143]. Reduced levels of miR-638 in serum EVs of HCC patients were negatively associated with tumor size, vascular invasion, and stage [144]. Current studies indicate that EVs found in the urine of patients with HCC are abundant in differentially expressed proteins, suggesting their potential utility in early non-invasive diagnosis [145]. These findings suggest that EVs could be valuable biomarkers for early HCC diagnosis, disease staging, and prognosis.

### *4.4. EVs as biomarkers for viral hepatitis*

Chronic liver disease caused by viral hepatitis remains a significant global health concern. Currently, clinical diagnosis of viral hepatitis primarily relies on viral antibodies like HBsAg and HAV-IgM. Recent research has indicated a higher expression of miR-19a in exosomes from HCV-infected hepatocytes and in the serum of patients with chronic HCV fibrosis [146]. Another study found reduced levels of miR204–5p, miR181a-5p, miR143–3p, miR93–5p, and miR122–5p in EV of HCV patients compared to healthy individuals. Interestingly, even post viral eradication through treatment, the levels of anti-fibrotic miRNAs miR204-p and miR143–3p in patient EVs did not recover, indicating a potential role of EVs in mediating persistent fibrosis in viral hepatitis [147]. Moreover, EVs released by HCV-infected hepatocytes carry HCV RNA, which binds to miR-122, Ago2, and HSP90 complexes, enhancing their stability and facilitating the spread of infection to naive hepatocytes. Consequently, serum EVs from HCV-infected patients contain a significant amount of HCV RNA, miR-122, Ago2, and HSP90, presenting a potential diagnostic utility [148].

## 5. Therapeutic potential of Hep-EVs

EVs injected into the body tend to accumulate in the liver, making them a promising treatment option for liver diseases [149]. Current focus in EVs therapy for liver diseases centers around mesenchymal stem cell therapy. Therapeutic application of Hep-EVs can be approached in two main ways. The first involves using engineered EVs as vehicles for drug delivery, such as lipid nanovesicles fused with EVs membranes for precise tumor targeting and efficient siRNA transfection [150]. The liver was reprogrammed to drive autonomous intrahepatic assembly and encapsulation of VEGFR2 siRNA into secreted EVs, enabling efficient in vivo VEGFR2 siRNA delivery [151]. Ye et al. encapsulated luteolin in Hep-EV, effectively addressing the issues of poor solubility, low bioavailability, and the lack of targeted delivery associated with luteolin [152].The second direction harnesses the protective properties of normal Hep-EVs, utilizing SK2 to promote liver regeneration and miR-423–5p to inhibit HSCs activation [46], thus potentially treating NAFLD [153] and fibrosis [154], and further studies indicate that this is due to the fact that the miR-423–5p they carry reduces the activation of HSCs [50]. Additionally, Hep-EVs have shown promise in reducing CRC invasion [52], treating neurodegenerative diseases [53], and preventing renal fibrosis [155]. This underscores the significant therapeutic potential of Hep-EVs, warranting further investigation.

## 6. Discussion and future perspectives

This article provides an overview of the impact of EVs released by hepatocytes on both the liver and extrahepatic organs in various physiological conditions. EVs form a crucial part of the liver's microenvironment, and alterations in their composition can offer insights into disease progression and underlying mechanisms. Recent research has broadened its focus beyond liver-specific pathologies, highlighting the role of EV-encapsulated cargo from hepatocytes in enhancing stability and facilitating communication between the liver and distant organs. Consequently, there is a growing interest in exploring the link between extrahepatic manifestations of chronic liver diseases and EVs. The proteins and nucleic acids carried by EVs released by healthy liver cells play a pivotal role in regenerative medicine. Despite the challenge of lower yields, normal Hep-EVs present a promising avenue for novel therapeutic interventions. Currently, there are no FDA-approved drug treatments for many liver diseases, such as NAFLD, ALD, etc. Consequently, molecular targeting associated with NAFLD has emerged as a significant trend in contemporary treatment strategies. However, clinical applications of Hep-EV still face several significant challenges. Among these,

the most critical issue is the absence of standardized methods for EV isolation, storage, and quality control. Furthermore, the identification of EV biomarkers requires additional support from clinical data. Additionally, inhibiting the biogenesis or release of EVs, or obstructing the uptake of EVs by other cells, represents a potential therapeutic approach to prevent the progression of the disease. Although numerous studies have demonstrated that Hep-EVs exhibit an increase in quantity and alterations in content under conditions of liver injury, the molecular mechanisms governing EV release and uptake remain unclear. Currently, the majority of research on the changes in the composition and content of Hep-EVs primarily focuses on miRNA, with relatively few studies addressing other substances carried within EVs, such as proteins, lipids, and transcription factors. Under conditions of liver injury, EVs secreted by hepatocytes exhibit an increase in quantity and changes in content. These elevated contents, such as miRNA and proteins, not only regulate other cells but also hold potential as clinical diagnostic markers. The authors anticipate that as research on EVs progresses, Hep-EVs will offer extensive applications in the clinical management and diagnosis of liver diseases.

## Abbreviations

ARF6, ADP-ribosylation factor 6; AH, alcoholic hepatitis; ALD, alcoholic liver disease; ASGPR, asialoglycoprotein Receptor; ABCA1, ATP-bindingcassettetransporterA1; CVD, cardiovascular disease; CRC, colorectal cancer; CRLM, colorectal cancer liver metastases; CXCL10, CXC motif ligand 10-bearing; CYR61, cysteine Rich Protein Angiogenic Inducer 61; CYTOR, cytoskeleton regulator RNA; DR5, death receptor 5; DKK1, dickkopf related protein 1; ESE, early endosomes; ESCRT, endosomal sorting complex required for transport; FA2H, fatty acid 2-hydroxylase; TFR, follicular regulatory T cells; FFA, free fatty acids; H2AFJ, H2A Histone Family Member; Hsp90, heat shock protein 90; HSCs, hepatic stellate cells; HCC, hepatocellular carcinoma; Hep-EVs, hepatocyte-derived EVs; IRE1α, inositol requiring enzyme 1α; KLF4, kruppel-like factor 4; LATS2, large tumor suppressor homolog 2; LSE, late endosomes; LRG1, leucine-rich alpha-2-glycoprotein 1; LSECs, liver sinusoidal endothelial cells; LAMP1,lysosomal associated membrane protein 1; LMP, lysosomal membrane permeability; LOXL4, lysyl Oxidase Like Protein 4; MASP1, mannose associated serine protease 1; MMP2, matrix metalloproteinase 2; RhoA, ras homolog gene family member A; MV, microvesicles; MVBs, multivesicular vesicles; NOX2, NADPH oxidase 2; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; Nrf2, nuclear factor erythroid-2-related factor 2; NF-κB, nuclear factor κb; NLRP3, nucleotide-binding domain leucine-rich repeat pyrin domain containing 3; OA, oleic acid; PA, palmitic acid; PPAR, peroxisome proliferator-activated receptor; PKM2, pyruvate kinase M2; RIP1, ras homolog gene family receptor interacting protein 1; RIG-I, retinoic acid inducible gene 1; RBP4, retinol-binding protein 4; ROCK1, rho-associated coiled-coil containing protein kinase 1; SELENOP, selenoprotein p; STAT6, signal transducer and activator of transcription 6; SK2, sphingosine kinase 2; S1P, sphingosine-1-phosphate; STARD11, StAR-related lipid transfer domain 11; SMEK1, suppressor of mek1; TLR9, toll like receptor 9; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TAMs, tumor-associated macrophages; VNN1, vanin-1; VEGFR2, vascular endothelial growth factor receptor 2; YAP, yes-associated protein; EVs, extracellular vesicles.

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## CRediT authorship contribution statement

**Honglei Shi:** Writing – review & editing. **Yongmin Yan:** Writing – review & editing. **Fang Cheng:** Writing – original draft. **Shihui Li:** Writing – original draft. **Ruizi Xu:** Writing – original draft. **Zhuan Zhang:** Writing – original draft.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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